Citation:

Dietrich M, Brown CJP, Block G. The effect of folate fortification of cereal-grain products on blood folate status, dietary folate intake and dietary folate sources among adult non-supplement users in the United States. *Journal of the American College of Nutrition* 2005; 24 (4): 266-274.

PubMed ID: 16093404

Study Design:

Trend study

Class:

D - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To explore the following:

- Changes in serum and erythrocyte folate status of the adult US population following folic acid fortification of enriched cereal-grain products
- Accompanying changes in food sources for folate and dietary total folate intake (including both food folate and folic acid).

Inclusion Criteria:

- Non-institutionalized US civilian
- Participant in National Health and Nutrition Examination Survey (NHANES) III (1988-1994) or NHANES 1999-2000
- Age greater than or equal to 20 years
- Non-pregnant
- Reported no supplement use in 30 days prior to dietary recall interview.

The IRB of the National Center for Health Statistics approved NHANES protocols.

Exclusion Criteria:

- Age less than 20 years
- Pregnant
- Reported supplement use in 30 days prior to dietary recall interview
- Unreliable dietary recall records as identified by NHANES.

Description of Study Protocol:

Recruitment

Stratified, multistage (counties, blocks and households) probability samples, including oversamples of some population subgroups:

- NHANES III: Young children, older adults, non-Hispanic blacks and Hispanics
- NHANES 1999-2000: Adolescents, older adults, pregnant women, non-Hispanic blacks, Hispanics and low-income persons.

Design

Both NHANES surveys are cross-sectional. NHANES III was conducted in two three-year phases (1988-1991 and 1991-1994), both of which were nationally representative. NHANES 1999-2000 contains nationally representative samples in each of the two years of data collection.

Participants completed physical examinations and dietary interviews in the NHANES mobile examination centers.

Dietary Intake/Dietary Assessment Methodology

- 24-hour dietary recalls; the interview protocols for the two survey waves were not described in detail in the report, as they are documented elsewhere.

Statistical Analysis

- Analyses were conducted SUDAAN to account for the complex sample design (i.e., weighting and variance estimation)
- Standard errors were estimated via the Taylor linearization for NHANES III and the jackknife procedure for NHANES 1999-2000
- Significance was determined using one-tailed Z-tests with $P \leq 0.01$ due to the large sample sizes.

Data Collection Summary:

Timing of Measurements

Subjects were assessed at a single timepoint, during which they completed the 24-hour dietary recall and a physical examination. NHANES III participants completed the survey in 1988-1994, prior to the mandatory folic acid fortification of cereal-grain products in 1998. NHANES 1999-2000 participants completed the survey after mandatory fortification was implemented.

Dependent Variables

- Serum folate concentrations (nmol/L)
- Erythrocyte folate concentrations (nmol/L)
- Dietary folate sources (23 categories; the report does not state how these categories were determined).

Prior to November 1993, serum and erythrocyte concentrations were measured via Quanta Phase I Folate Radioassay Kit. After November 1993, the Quanta Phase II Folate Radioassay Kit was used. The CDC applied a correction factor to account for differences between the two kits (the Phase I kit had contained incorrect calibrator solutions).

Independent Variables

- Mandatory folic acid fortification of cereal-grain products in 1998
- Dietary folate intake (mcg per day).

Description of Actual Data Sample:

Initial N

NHANES III contained a total of 17,158 subjects and NHANES 1999-2000 contained a total of 8,843 subjects. After

applying exclusion criteria the final sample size was:

Survey	N
NHANES III	9,919
NHANES 1999-2000	2,121

Attrition (final N)

After applying inclusion or exclusion criteria, the authors noted that blood folate concentrations were available for the following number of cases:

Available folate data		
Survey	Serum	Erythrocyte
NHANES III	9,430	9,438
NHANES 1999-2000	1,978	2,007

Age

Age					
Survey	Sex	20-39 years	40-59 years	60+ years	Total
NHANES III	Males	2,199	1,359	1,517	5,075
	Females	2,260	1,302	1,282	4,844
	Total	4,459	2,661	2,799	9,919
NHANES 1999-2000	Males	430	323	393	1,146
	Females	356	305	314	975
	Total	786	628	707	2,121

Ethnicity

Breakdowns were not reported, but both surveys included oversamples of minority groups and were weighted to be nationally representative.

Location

US.

Summary of Results:

- Both serum and erythrocyte folate concentrations increased significantly (P<0.0001 for all age-sex groups) post-fortification
- The mean serum folate concentration increased more than two-fold in the overall study population (136%), from 11.4nmol/L to 26.9nmol/L
- Mean erythrocyte folate, a marker of long-term folate status, increased 57% overall, from 375nmol/L to 590nmol/L in the overall study population
- RBC folate concentrations increased by 63% in women aged 20-39 and 40-59 years
- The mean total folate intake of the study population increased by 76mcg per day (28%), from 275mcg per day to 351mcg per day
- Dietary total folate intake also increased significantly in all age-sex groups (P<0.01), except females age 60 and older (P=0.18), with the largest increase among women age 20 to 39.

	Serum folate (nmol/L mean±SEM)			Erythrocyte folate (nmol/L mean±SEM)			Dietary total folate intake (mcg per day mean±SEM)		
	NHANES III	NHANES 1999-2000	Percentage change	NHANES III	NHANES 1999-2000	Percentage change	NHANES III	NHANES 1999-2000	Percentage change
Total	11.4±0.24	26.9±0.49	+136	375±3.8	590±11.6	+57	275±3.2	351±9.1	+28
Males	11.1±0.27	25.8±0.58	+133	371±4.3	578±13.1	+56	315±3.8	401±13.0	+27
20-39 years	9.9±0.25	24.0±0.67	+143	346±4.6	533±9.8	+54	316±6.2	410±15.7	+30
40-59 years	11.1±0.47	26.4±0.91	+139	372±7.1	616±23.1	+66	315±8.3	409±18.6	+30
60 years and older	14.3±0.48	30.9±1.51	+116	440±9.1	655±30.5	+49	313±8.7	356±16.1	+14
Females	11.8±0.32	28.3±0.69	+139	380±5.5	603±15.1	+59	230±5.2	292±9.8	+27
20-39 years	10.3±0.28	26.0±1.07	+153	341±6.2	556±16.1	+63	217±5.2	294±12.6	+36
40-59 years	11.4±0.41	27.1±1.04	+137	386±9.8	629±21.6	+63	232±14.4	302±15.8	+30
60 years and older	15.6±0.66	36.4±1.59	+134	456±8.8	686±24.6	+51	255±7.7	270±15.4	+6

Other Findings

- Prevalence of inadequate serum folate (less than 7nmol/L) and erythrocyte folate (less than 305nmol/L) declined by 25% and 35%, respectively (P<0.0001) between the two surveys
- Fortified foods (bread including rolls and crackers, rice and other grain products and pasta) became the largest folate sources post-fortification. Vegetables and breakfast cereals, the top sources in NHANES III, became the second and third largest total dietary folate contributors behind bread.

Author Conclusion:

- Food fortification more than doubled the mean serum folate status of the total adult US population and mirrors the findings of other published studies
    - Women of childbearing age are now well exceeding the lower limit of the acceptable range of serum folate levels (≥13.6nmol/L) associated with very low risk for neural tube defects
- Fortification has significantly improved the median erythrocyte folate status in the total US population
    - More than 90% of women of childbearing age did not reach erythrocyte folate levels of the recommended 906nmol/L, a level associated with a significant reduction of neural tube defects. These women should take folic acid supplements in order to reach recommended folate levels.
- Total dietary folate intake in the total population increased 28% post-fortification. With substantially higher serum levels, the discrepancy likely reflects an underestimation of dietary folate intake because of the nutrient databases used to convert the recall interviews to nutrients. Using regression equations developed by other researchers, it is estimated that total folate intake in some of the population may exceed the Tolerable Upper Intake Level for folic acid; long-term effects and safety of the fortification policy must be monitored
- As expected, the ranking of food sources contributing to dietary folate intake changed post-fortification, with fortified grain products becoming more important sources.

Reviewer Comments:

- *The authors noted that the food composition tables used for the NHANES database do not distinguish between naturally occurring food folate and synthetic folate acid, so that intake in dietary folate equivalents cannot be determined. Similarly, intakes in the study also underestimate the post-fortification increase in folate*
- *Although the authors believe that changes in folate status are very likely due to fortification rather than changing eating behaviors between the surveys, no competing explanations (i.e., history threats to internal validity) were raised or discussed*
- *Excluding supplement users was necessary because analyzable supplement data were not available in NHANES 1999-2000. However, the large number of subjects excluded based on this criterion may limit generalizability.*
- *Although blood folate concentrations were not available on all subjects included in the study, the reduction of sample size was less than 10%, which is within the acceptable range for missing data according to the National Center for Health Statistics NHANES tutorials. However, it is possible that subjects with these data available may be different from those for whom the data were missing, which may bias the findings*
- *Since appropriate weighting and variance estimation techniques were used to properly account for the complex sample design, each NHANES wave was nationally representative of the US non-institutionalized population. Population demographics were likely to have changed in the years between the surveys, so the two comparison groups are not strictly comparable in the meaning intended in the Research Design and Implementation Rating Checklist.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	N/A
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	N/A

Validity Questions		
1.	**Was the research question clearly stated?**	Yes

1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	No
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	???
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	N/A
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	N/A
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	N/A
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	???
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	???
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	No
4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes

5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	Yes
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	N/A
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	Yes
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	Yes
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	N/A
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	???
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes

	8.6.	Was clinical significance as well as statistical significance reported?	Yes
	8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**		Yes
	9.1.	Is there a discussion of findings?	Yes
	9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**		Yes
	10.1.	Were sources of funding and investigators' affiliations described?	Yes
	10.2.	Was the study free from apparent conflict of interest?	Yes